Food and Chemical Toxicology 127 (2019) 72–80

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Mkp-1 is required for chemopreventive activity of butylated hydroxyanisole and resveratrol against colitis-associated colon tumorigenesis

Zhaohong Zheng[a], Yeru Chen[a,1], Jianan Huang[b,c], Hong Deng[d], Xiuwen Tang[c], Xiu Jun Wang[a,*]

[a] Department of Pharmacology and Cancer Institute of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310058, PR China
[b] Chu Kochen Honors College, Zhejiang University School of Medicine, Hangzhou, 310058, PR China
[c] Department of Biochemistry, Department of Thoracic Surgery of the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310058, PR China
[d] Department of Pathology, Zhejiang University School of Medicine, Hangzhou, 310058, PR China

A R T I C L E I N F O

Keywords:
Mitogen-activated protein kinase phosphatase 1
Nrf2
Chemopreventive agent
Colitis-associated tumorigenesis

A B S T R A C T

Many dietary compounds show promising protective activity against colon cancer by activating nuclear factor-erythroid 2 related factor 2 (Nrf2). Recently, we reported that mitogen-activated protein kinase phosphatase 1 (Mkp-1) exhibits crosstalk with the Nrf2 signaling pathway, protecting against intestinal inflammation. Here, we present evidence that Mkp-1 is required for the chemopreventive action of the Nrf2 activators butylated hydroxyanisole (BHA) and resveratrol (RSV). In an azoxymethane/dextran sulfate sodium model of colitis-associated tumorigenesis, *Mkp-1*$^{-/-}$ mice exhibited a phenotype similar to *Nrf2*$^{-/-}$ mice with significantly more tumors than WT mice. Tumors from *Mkp-1*$^{-/-}$ mice exhibited higher levels of macrophage infiltration than those from WT mice. This was accompanied by increased expression of nitrotyrosine and p53BP1, markers of oxidative stress and DNA damage, respectively. Moreover, dietary suppression of tumorigenesis using BHA (0.5%) or RSV (300 ppm) supplementation was achieved in WT but not in *Mkp-1*$^{-/-}$ mice. In adenomas from WT mice, the expression of Mkp-1 was markedly lower than in adjacent normal tissue, concomitant with the down-regulation of Nrf2 and its target genes. Our data revealed that Mkp-1 is required in the protective role of Nrf2 signaling against colitis-associated tumorigenesis.

1. Introduction

Colorectal cancer is one of the most common types of cancer worldwide (Siegel et al., 2013). Chronic inflammation is recognized as a potential risk factor for this and other cancers. People with inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are more prone to colorectal cancer (Eaden et al., 2001; Friedman et al., 2001). Therefore, there is an urgent need to develop novel chemopreventive interventions for this high-risk population.

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a transcription factor that controls both the basal and inducible expression of a battery of cytoprotective genes, including the antioxidant and phase II drug-metabolizing enzymes glutathione transferases (GSTs), NAD(P)H:quinone oxidoreductase 1 (NQO1), glutamate-cysteine ligase (GCLC), catalytic subunit, and heme oxygenase-1 (HO-1) (Itoh et al., 1997). At least ten chemical classes of Nrf2 activators have been identified (Dinkova-Kostova et al., 2004; Holtzclaw et al., 2004; Kensler and Wakabayashi, 2010). Under normal redox homeostatic conditions, cytoplasmic Kelch-like ECH-associated protein-1 (Keap1) physically binds Nrf2, leading to its proteasomal degradation *via* cullin 3 ubiquitination (Dinkova-Kostova and Talalay, 2008). Upon exposure to inducers, Keap1 interacts with inducers resulting in conformational changes that abrogate the capacity of Keap1 to inhibit Nrf2. Nrf2 is translocated to the nucleus, and dimerizes with small Maf (musculoaponeurotic fibrosarcoma) proteins, binds to antioxidant response element (AREs), and activates the transcription of its target genes.

Nrf2 is recognized as a drug target for the prevention of colorectal cancer. Nrf2-deficient mice are more susceptible to dextran sulfate

Abbreviations: AOM, azoxymethane; ARE, antioxidant response element; BHA, butylated hydroxyanisole; DSS, dextran sulfate sodium; Gclc, glutamate-cysteine ligase, catalytic subunit; Gst, glutathione transferase; Ho-1, heme oxygenase 1; IHC, immunohistochemistry; Keap1, Kelch-like ECH-associated protein 1; Mkp-1, mitogen-activated protein kinase phosphatase 1; Nrf2, nuclear factor-erythroid 2 related factor 2; Nqo1, NAD(P)H:quinone oxidoreductase 1; RSV, resveratrol; RT-qPCR, real-time quantitative PCR; WT, wild-type

* Corresponding author. Department of Pharmacology and Cancer Institute of Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310058, PR China.

E-mail address: xjwang@zju.edu.cn (X.J. Wang).

[1] Current address: Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou 310016, PR China.

https://doi.org/10.1016/j.fct.2019.02.044
Received 11 October 2018; Received in revised form 4 February 2019; Accepted 27 February 2019
Available online 04 March 2019

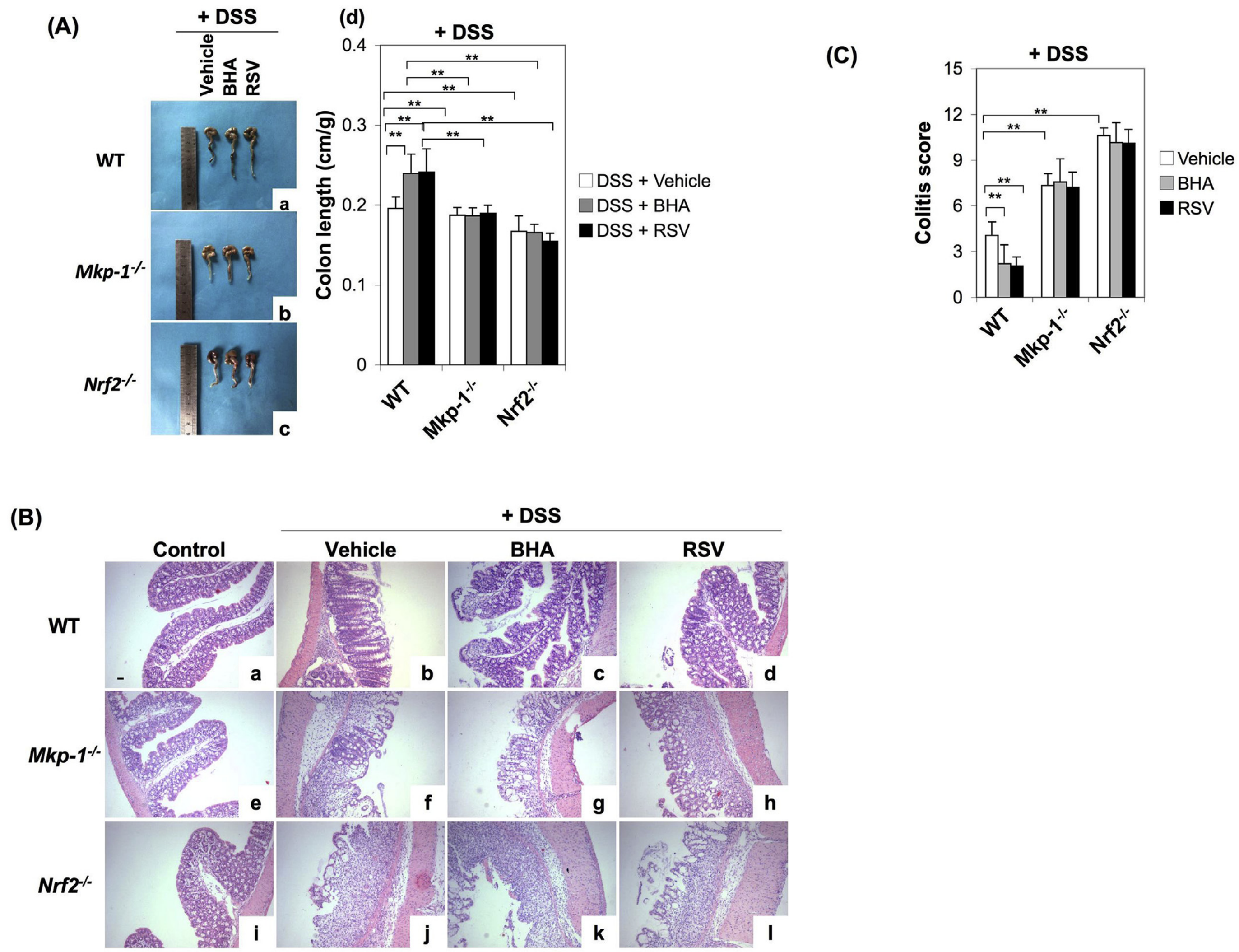

Fig. 1. Mkp-1 knockout abolishes the anti-colitis effect of BHA and RSV. WT, *Mkp-1*$^{-/-}$, and *Nrf2*$^{-/-}$ mice were given BHA (200 mg/kg i.g.), RSV (200 mg/kg i.g.), or vehicle (corn oil) daily, starting 2 days before DSS administration (2.5%) in drinking water for 1 week, until the termination of the experiment after 7 days of DSS treatment. (**A**) Representative images of the colon (a–c) and statistics for colon length (d) from mice treated with DSS + Vehicle, DSS + BHA, or DSS + RSV. Colon length is represented as a ratio (cm/g) compared to the starting weight of mice prior to DSS administration (n = 8 [WT]; 6 [*Mkp-1*$^{-/-}$]; 4 [*Nrf2*$^{-/-}$]). (**B**) Representative images of H&E-stained colon sections. Original magnification × 40; scale bar, 100 μm; a – d, WT; e – h, *Mkp-1*$^{-/-}$; i – l, *Nrf2*$^{-/-}$. Control, mice given normal drinking water without any treatment. (**C**) Colitis scores (n = 8 [WT]; 6 [*Mkp-1*$^{-/-}$]; 4 [*Nrf2*$^{-/-}$]; mean ± SEM; **P < 0.01 *vs* WT mice treated with DSS + Vehicle).

sodium (DSS)-induced inflammation/colitis and to colitis-associated colorectal cancer (Khor et al., 2006, 2008; Osburn et al., 2007). Many natural and synthetic compounds have been reported to suppress colitis-associated carcinogenesis in animal models by activating the Nrf2/ARE signaling pathway (Reimund et al., 1998; Gescher et al., 2001; Greenwald et al., 2002; Martin et al., 2006; Cheung et al., 2010; Cui et al., 2010; Krehl et al., 2012; Lippmann et al., 2014; Yang et al., 2014; Long et al., 2015; Pandurangan et al., 2015; Wu et al., 2017). Although Keap1 is well established as the sensor in the cytoplasm for these activators, other proteins that may be involved in the activation of Nrf2 by the these inducers remain elusive.

Mitogen-activated protein kinase phosphatase 1 (Mkp-1; also named dual-specific phosphatase 1, DUSP1), is a nuclear phosphatase and a key negative regulator in the innate immune response (Liu et al., 2007). By dephosphorylating threonine and tyrosine, this phosphatase deactivates p38 and c-Jun N-terminal kinases, which play pivotal regulatory roles in the biosynthesis of proinflammatory cytokines (Liu et al., 2007). Recently, we reported that Mkp-1 is implicated in the regulation of the Nrf2/ARE cytoprotective system *via* crosstalk with Nrf2 (Luo et al., 2018; Li et al., 2018). Through a direct interaction with the Neh2 domain of Nrf2, Mkp-1 stabilizes Nrf2, leading to increased activity of the transcription factor and upregulation of its downstream genes. Conversely, Nrf2 activates Mkp-1 transcription by binding to the ARE in the promoter of *Mkp-1*. Mkp-1 is involved in the regulation of both the basal and inducible expression of ARE-driven genes, and has been implicated in the protective action of the Nrf2 activators butylated hydroxyanisole (BHA) and sulforaphane against liver injury (Luo et al., 2018). A role of the Mkp-1/Nrf2 axis in limiting inflammation in DSS-induced murine colitis has also been demonstrated (Li et al., 2018). Based on the importance of Mkp-1 in regulating the Nrf2 signaling pathway, we hypothesized that Mkp-1 may play a key role in the action of Nrf2 activators in reducing the risk for colon tumorigenesis.

BHA is a synthetic phenolic antioxidant that is widely used as an antioxidant and preservative in food, food packaging, and medicines. Resveratrol (RSV) is a dietary polyphenolic compound present in many edible plants. Both agents are well-characterized Nrf2 activators that have protective effects against inflammation and carcinogenesis in humans and in animal models (Talalay et al., 1978; Pearson et al.,

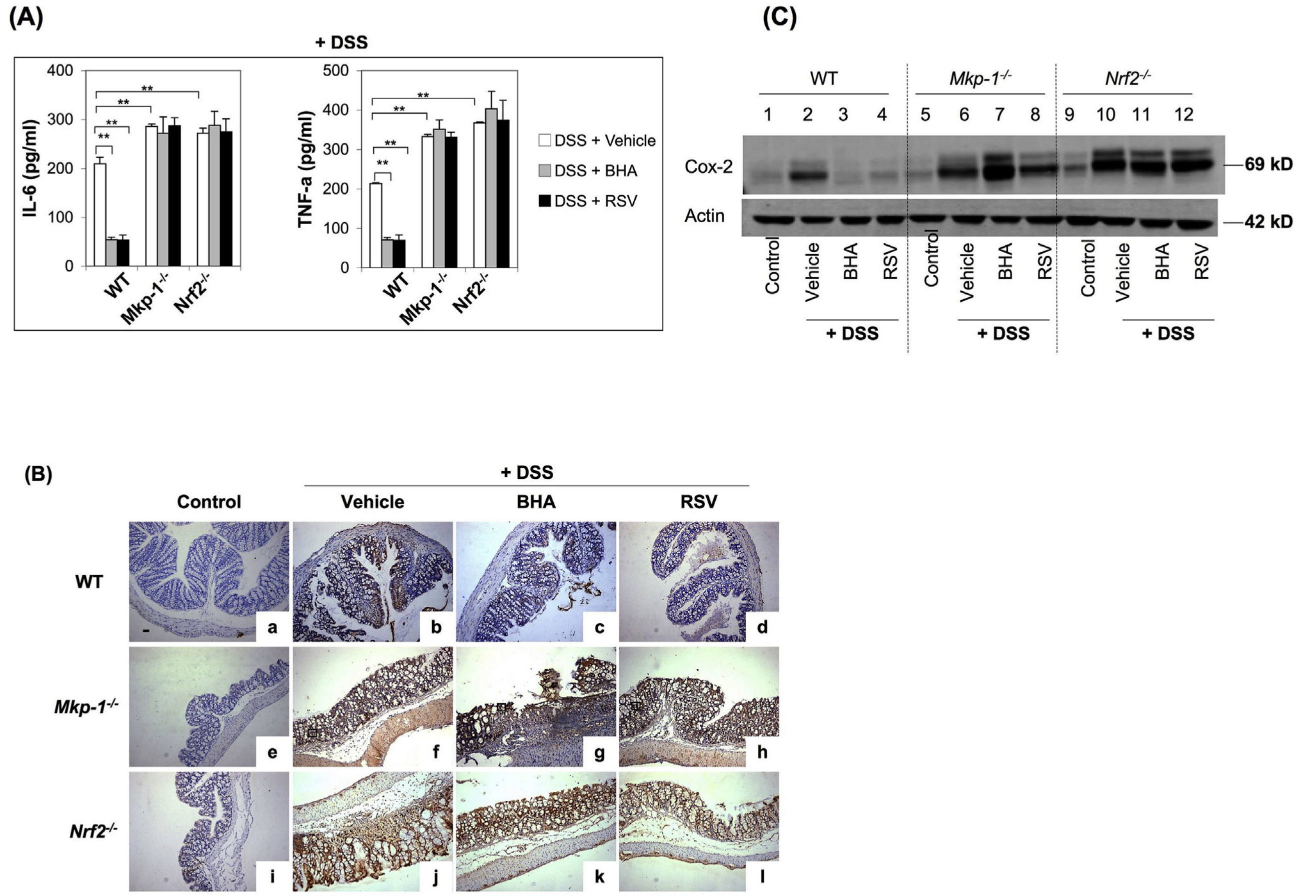

Fig. 2. Mkp-1 knockout abolishes the inhibitory effect of BHA and RSV on inflammation and oxidative stress in murine DSS-induced colitis. WT, *Mkp-1*$^{-/-}$, and *Nrf2*$^{-/-}$ mice were given BHA (200 mg/kg i.g.), RSV (200 mg/kg i.g.), or vehicle (corn oil) daily, starting 2 days before DSS administration (2.5%) in drinking water for 1 week, until the termination of the experiment after 7 days of DSS treatment. (**A**) Serum IL-6 and TNF-α levels of mice treated with DSS + Vehicle, DSS + BHA, or DSS + RSV (mean ± SEM; **P < 0.01 *vs* WT mice treated with DSS + Vehicle). (**B**) Representative nitrotyrosine immunostaining of colon sections from mice without treatment (control) (a, e, i); treated with DSS + vehicle (b, f, j); treated with DSS + BHA (c, g, k); or treated with DSS + RSV (d, h, l). Original magnification × 40; scale bar, 100 μm. (**C**) Typical Western immunoblots for anti-Cox2 in cell lysates from the colon of mice with or without treatment. Actin served as a loading control. Each lane represents lysate from a single mouse. The examples are from at least three separate experiments.

1983; Wattenberg, 1985; Itoh et al., 1997; Rubiolo et al., 2008; Li et al., 2014; Singh et al., 2014; Elshaer et al., 2018). We therefore used BHA and RSV in this study as prototype Nrf2 activators to investigate the chemopreventive role of Mkp-1. In a mouse model of azoxymethane (AOM)/DSS-induced colitis-associated tumorigenesis, we present evidence that Mkp-1 is essential for the suppression of colonic tumorigenesis by BHA and RSV.

2. Materials and methods

2.1. Chemicals and antibodies

Unless otherwise stated, all chemicals were from Sigma-Aldrich Co., Ltd (St. Louis, MO, USA). Antibodies were from Santa Cruz Biotechnology (Dallas, TX, USA). DSS (36–50 kD) was from MP Biomedicals (Aurora, OH, USA). Antibodies against mouse Nrf2 (H300; sc-13032) and Mkp-1 (C-19) were from Santa Cruz Biotechnology. Antisera against Ho-1, Nqo1, and Gclc were generated in our laboratory (Luo et al., 2015a, 2015b). Antiserum against Gstα1/2 was kindly provided by Professor John Hayes (University of Dundee, UK).

2.2. Animals

BALB/c background wild-type (WT) mice were purchased from Shanghai Laboratory Animal Center (Chinese Academy of Sciences, Shanghai, China). C57BL/6 background *Nrf2*$^{-/-}$ mice were kindly provided by Prof. Masayuki Yamamoto (University of Tsukuba, Japan) (Itoh et al., 1997). With the permission of Bristol-Myers Squibb Co. (New York, NY, USA), *Mkp-1*$^{-/-}$ mice on the C57BL/6 background were kindly provided by Professor Andrew R. Clark (University of Birmingham, UK) (Smallie et al., 2015). BALB/c background *Nrf2*$^{-/-}$ and *Mkp-1*$^{-/-}$ mice were produced by 8 back-crossings of C57BL/6 background *Nrf2*$^{-/-}$ and *Mkp-1*$^{-/-}$ mice with BALB/c WT mice. Genotypes of *Nrf2*$^{-/-}$ and *Mkp-1*$^{-/-}$ mice were routinely determined by RT-PCR and confirmed by Western immunoblotting. All animal procedures were performed with the approval of the Laboratory Animal Ethics Committee of Zhejiang University.

2.3. Colitis and colitis-associated tumorigenesis

To induce colitis, 6- to 8-week-old male C57BL/6 background mice were given 2.5% DSS in the drinking water *ad libitum* for 7 days. Mice given regular drinking water throughout the experimental period were used as controls. The dose of BHA used in this study is same as that used in the previous studies from our laboratory (Luo et al., 2015a, 2018) and others laboratories (Hu et al., 2006; Nair et al., 2006). Previous studies have shown that RSV doses in the range of 15–750 mg/kg in the mouse are effective in delaying or preventing carcinogenesis without any toxicity (Gescher and Steward, 2003). A dose of 200 mg/kg RSV was chosen for this study. In BHA and RSV treatment experiments, male

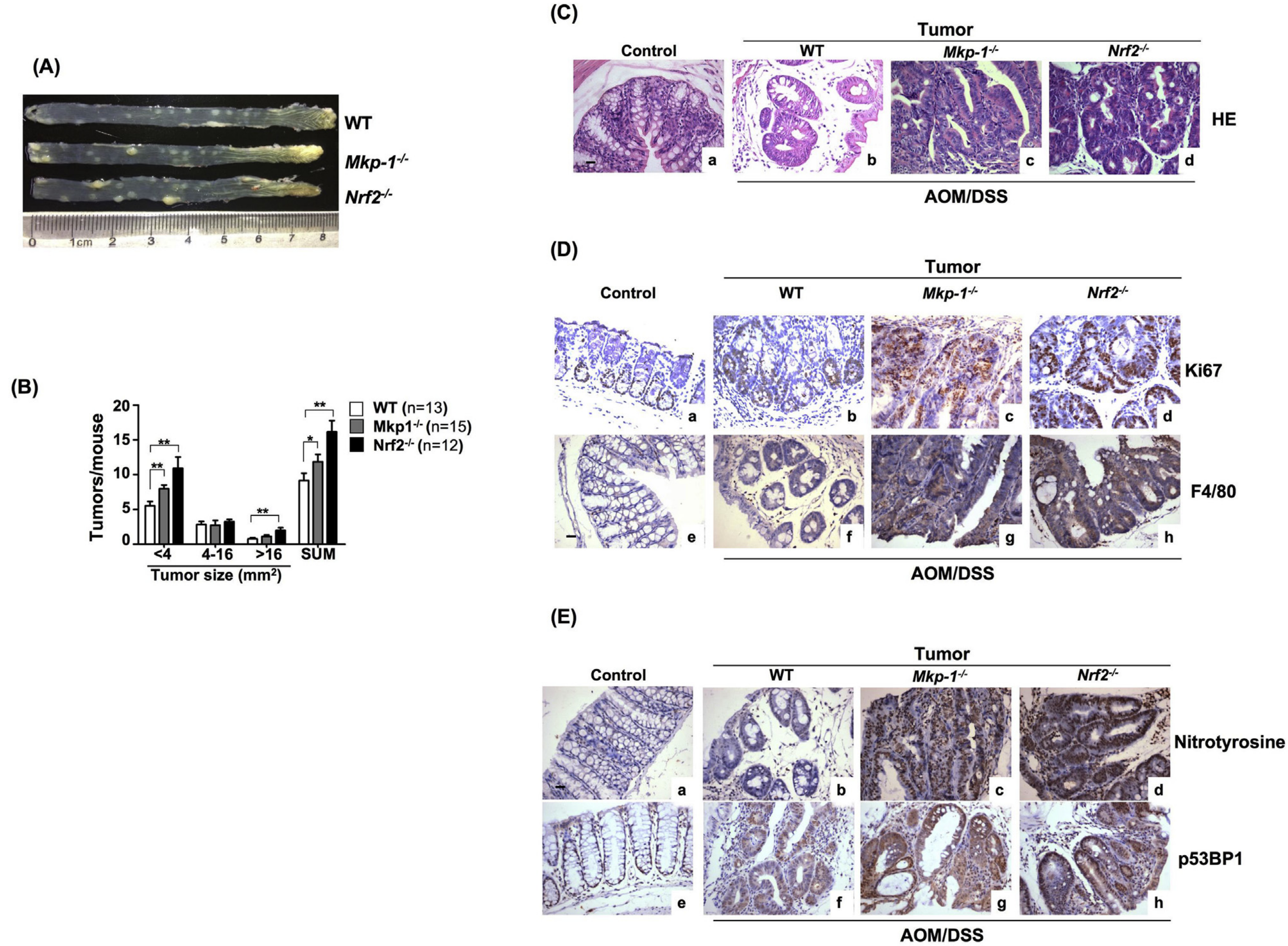

Fig. 3. Mkp-1 deficiency exacerbates adenoma formation after AOM/DSS treatment. BALB/c WT, *Mkp-1*$^{-/-}$, and *Nrf2*$^{-/-}$ mice were subjected to 6-weeks AOM/DSS treatment to induce colitis-associated tumorigenesis. **(A)** Photographs of colonic adenomas from mice after AOM/DSS induction. **(B)** Size distribution (left) and total number (SUM, right) of colonic tumors per mouse (n = 13 [WT]; 15 [*Mkp-1*$^{-/-}$]; 12 [*Nrf2*$^{-/-}$]). Values are mean ± SEM; *P < 0.05, **P < 0.01 *vs* WT mice. **(C)** Representative H&E staining of colonic tumor sections at the end of AOM/DSS treatment (a–d). Control, normal colon sections from WT mice without any treatment. Scale bar, 100 µm. Original magnification × 200. (**D** and **E**) Mkp-1 deficiency exacerbates inflammation and oxidative stress in colon from mice exposed to AOM/DSS. **(D)** Representative IHC staining for anti-ki67 (a–d) and anti-F4/80 (e–h) in colonic tumor sections. **(E)** Representative IHC staining for anti-nitrotyrosine (a–d) and p53BP1 (e–h) in colonic tumor sections. Control, normal colon sections from WT mice without treatment. Scale bar, 100 µm. Original magnification × 200.

C57BL/6 background mice (6–8 weeks old) were given BHA (200 mg/kg i.g.), RSV (200 mg/kg i.g.), or vehicle (corn oil) daily, starting 2 days before the administration of DSS (2.5%) in the drinking water for one week, after which the experiment was terminated and the mice were killed by cervical dislocation. The colon was processed as described previously (Li et al., 2018). Tissue sections (3 µm) were stained with hematoxylin and eosin (H&E) as previously described (Khor et al., 2006). The histological score was the sum of the scores for four parameters: inflammation severity (0, 1, 2, or 3), ulceration (0 or 1), area of inflammation (0, 1, 2, 3, or 4), and hyperplasia and dysplasia (0, 1, 2, or 3) as detailed previously (Pascal, 1994).

Colitis-associated adenoma was induced following a six-week AOM/DSS treatment protocol modified from that of Suzuki et al. (2004). Briefly, 5–6-week-old male BALB/c mice were given a single injection of AOM (10 mg/kg i.p.) once per week for the first three weeks, and exposed to drinking water containing 1.5% DSS. This was followed by normal drinking water for 3 weeks. Throughout, the mice were monitored for body weight, diarrhea, and hematochezia. The protocol for the effect of BHA or RSV supplementation on tumorigenesis is summarized in Fig. 4A. The WT mice were fed an AIN-93M diet supplemented with BHA (0.5%) or RSV (300 ppm) one week prior to the AOM/DSS treatment as described above until the end of the experiment. Mice fed the AIN-93M diet and receiving AOM/DSS served as controls. Colons were processed as described above.

2.4. Western blot analysis, H&E staining and immunohistochemistry (IHC)

The procedures for protein extraction from colon tissue and Western immunoblotting are provided elsewhere (Luo et al., 2015a, 2015b). Briefly, soluble proteins were extracted in a lysis buffer containing 10 mM Tris HCl pH 8.0, 50 mM NaCl, 50 mM NaF, 100 mM Na_3VO_4, 5 mM $ZnCl_2$, 0.5% Tirton X-100 and protease inhibitors. The protein samples were separated by SDS-PAGE and transferred from the gel to PVDF membrane (ThermoFisher Scientific, Beijing, China). The membrane was blocked for 1 h at room temperature using the blocking buffer containing 10% non-fat dry milk in TBST before incubation with diluted primary antibody in the blocking buffer overnight at 4 °C. Anti-cox-2, anti-Mkp-1, anti-Nrf2, anti-Ho-1 and anti-gclc were at 1/1000 dilution, anti-Nqo1 at 1/2000, and anti-gsta1/2 at 1/5000 dilution. After three washes of TBST, the membrane was incubated with diluted secondary HRP-conjugated antibody (Zhongshan Golden Bridge Biotechnology, Beijing, China) in blocking buffer at room temperature for 1 h. After three washes of TBST, the signal was developed using SuperSignal™ Western Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific) by following the manufacture's instruction. Immunoblotting with antibody against actin was performed to confirm

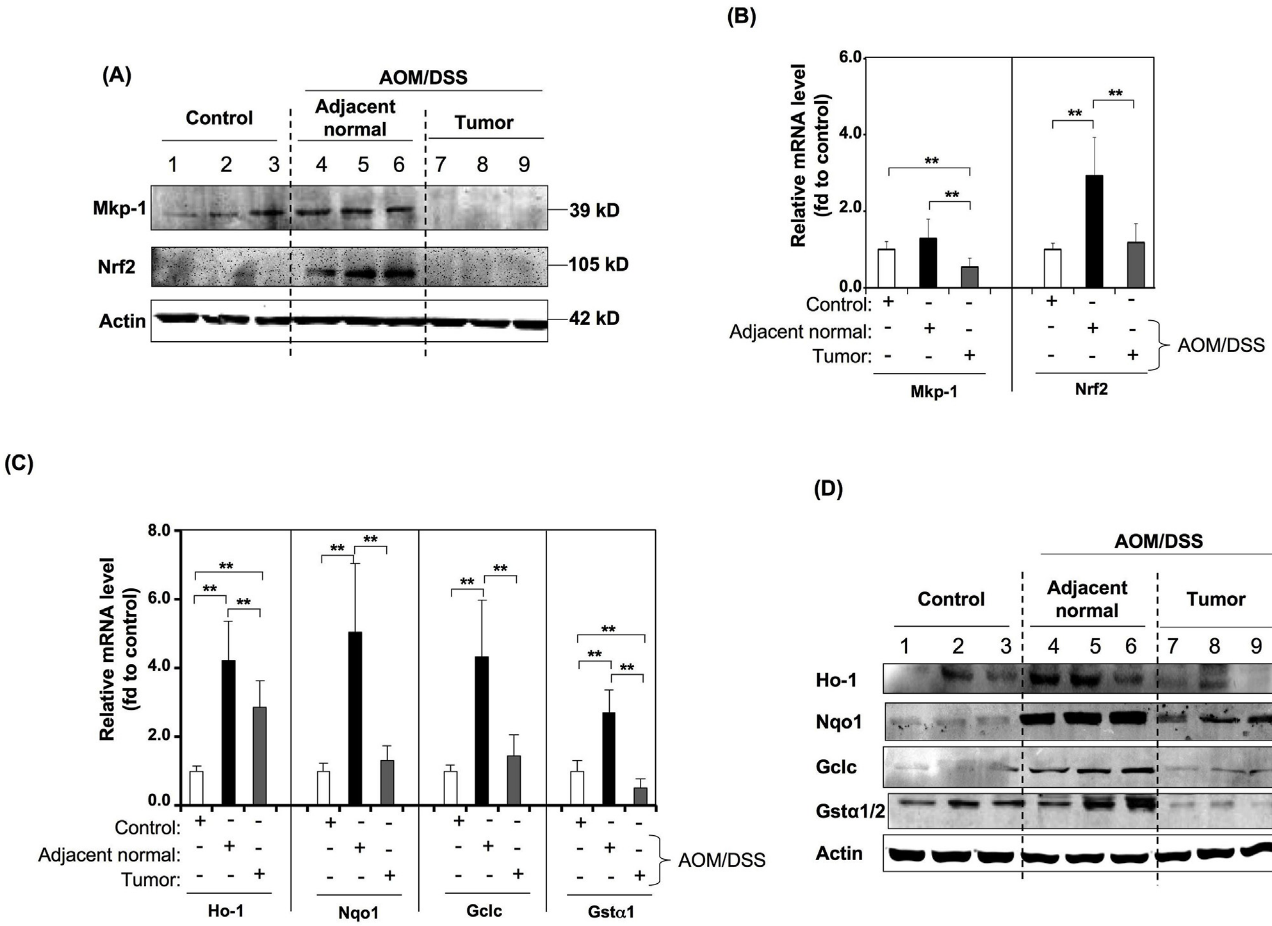

Fig. 4. Down-regulation of Mkp-1, Nrf2 and ARE-driven genes in adenoma compared to adjacent normal mucosa. BALB/c WT were subjected to 6-weeks AOM/DSS treatment to induce colitis-associated adenoma. The adenoma and the adjacent normal colon tissue were harvested separately. (**A**) Typical Western immunoblots for anti − Mkp-1 and anti − Nrf2 in tissue lysate from adenomas and in adjacent normal mucosa in the colon of WT mice exposed to AOM/DSS. Actin was used as a loading control. For control and adjacent normal tissue, each lane represents lysate from a single mouse. For adenoma samples, each lane represents lysate from a pooled sample of 3–5 tumors. Control, normal WT mice without treatment. **(B** and **C)** mRNA levels of Mkp-1, Nrf2, Ho-1, Nqo1, Gclc, and Gstα1 in adenomas and in adjacent normal mucosa in the colon of WT mice exposed to AOM/DSS. For control and adjacent normal tissue, each sample represents RNA from a single mouse. For adenomas, each sample represents RNA from a pooled sample of 3–5 tumors. Control, normal WT mice without treatment. 18S RNA was used as internal control. The value for control mice was set at 1 (mean ± SEM, n = 3; **P < 0.01). (**D**) Representative Western immunoblots of Ho-1, Nqo1, Gclc, and Gstα1 in adenomas and in adjacent normal mucosa in colon from WT mice exposed to AOM/DSS. Tissue lysates were prepared separately from the adenoma and the adjacent normal tissue from mice colon developed adenoma, and subjected to Western immunoblotting analysis with antibody against Ho-1, Nqo1, Gclc, or Gstα1. Actin was used as a loading control. For control and adjacent normal tissue, each lane represents lysate from a single mouse. For adenoma samples, each lane represents lysate from a pooled sample from 3–5 tumors. Control, normal WT mice without treatment.

equal loading for whole-cell extracts.

H&E staining and IHC of colon sections from mice was carried out using formalin-fixed paraffin-embedded tissue as described previously (Luo et al., 2015a, 2015b). Briefly, Tissues were fixed with 10% formaldehyde in PBS, embedded in paraffin, sectioned into 4-μm thick sections and mounted on glass slides for immunohistochemical analysis. Sections were deparaffinized in xylene and rehydrated in a series of graded ethanol. The endogenous peroxidase activity of the specimens was blocked by immersing the slides in a 3% hydrogen peroxidase–methanol solution for 10 min at room temperature. After three washes of PBS, sections were pretreated with citrate buffer for 2 min at 121 °C in a microwave oven to retrieve antigenicity. To block non-specific reactions, the sections were treated with 1% bovine serum albumin in PBS for 60 min at room temperature. The sections were next incubated overnight with diluted primary antibody at 4 °C. Anti-nitrotyrosine and anti-F4/80 were at 1/200 dilution, anti-ki67 at 1/300, and anti-p53bp1 at 1/1000 dilution. After three washes of PBS, the sections were incubated with diluted secondary HRP-conjugated antibody (Zhongshan Golden Bridge Biotechnology) in a humidity chamber for 30 min at room temperature. After three washes of PBS, sections were stained with DAB (3,3′-Diaminobenzidine) solution (HuaAn Biotechnology, Hangzhou, China) by following the manufacture's instruction, and counterstained with hematoxylin. Finally, the sections were dehydrated, cleared and mounted. IHC staining was evaluated by two independent pathologists, who were blinded to the treatments. For each mouse, three separate slides were analyzed. Five microscopic fields in tissues were randomly selected and evaluated.

2.5. Real-time quantitative PCR (RT-qPCR)

Total RNA isolation and RT-qPCR were performed as described previously (Wang et al., 2006). The primers and probes were synthesized by TaKaRa Biotechnology (Dalian, China). qPCR using the validated SYBR® Green or TaqMan assays were carried out on a LightCycler® 480 instrument (Roche, Mannheim, Germany). The primers and probes for mouse Nqo1, Gclc, and Gstα1 were as described previously (Luo et al., 2018). The sequences of the primers and probes for measuring mouse Ho-1 and Mkp-1 mRNAs were as provided elsewhere (Li

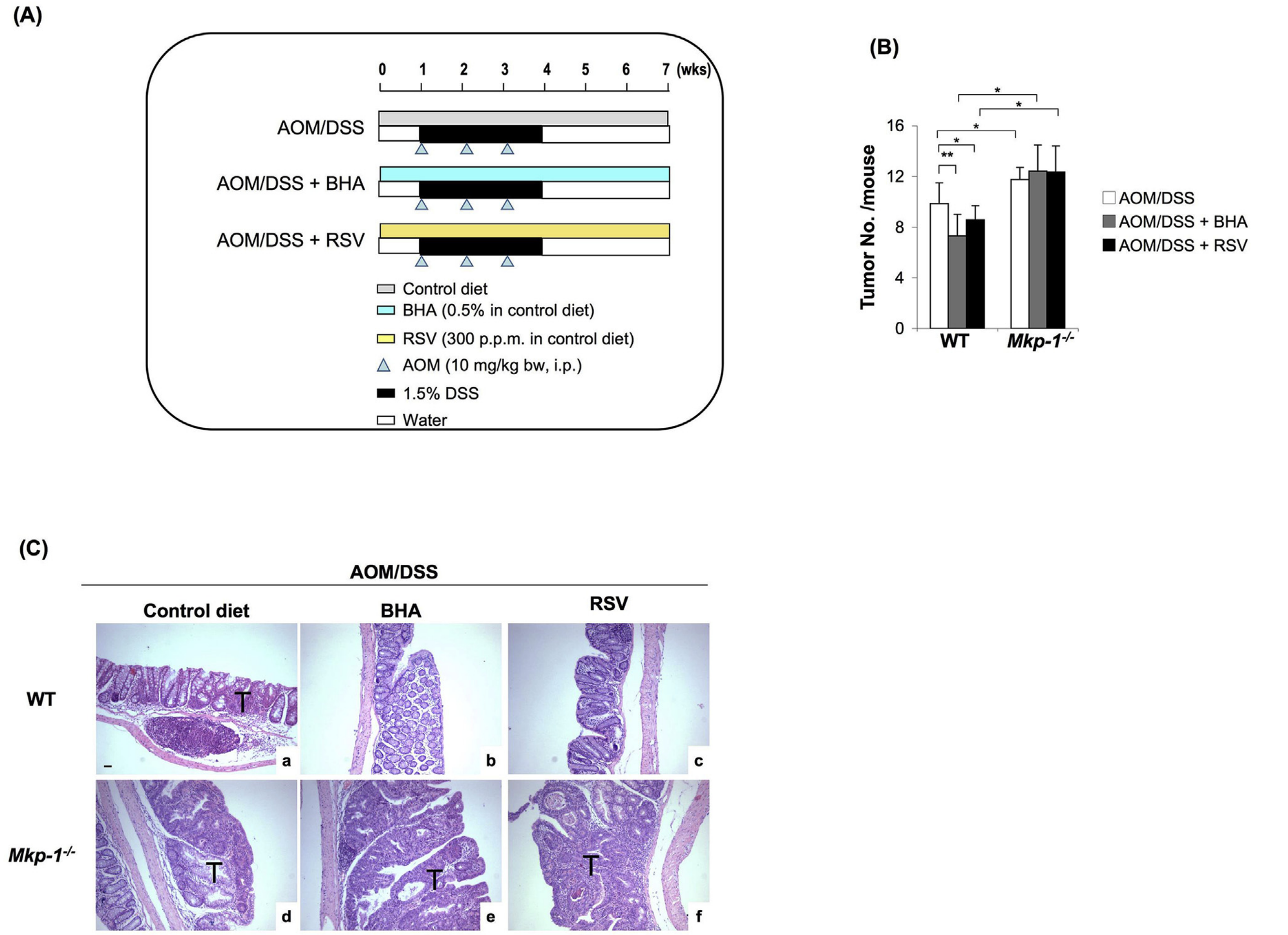

Fig. 5. Mkp-1-knockout abolishes the chemopreventive action of BHA and RSV on colitis-associated tumorigenesis. (A) Experimental protocols for assessing the effect of BHA and RSV on colitis-associated tumorigenesis. BALB/c WT and *Mkp-1*$^{-/-}$ mice were subjected to 6-weeks AOM/DSS treatment to induce colitis-associated tumorigenesis. The mice were fed with the control diet or the diet supplemented with BHA (0.5%) or RSV (300 ppm) one-week prior to the AOM/DSS treatment, and continued through the whole experimental period. (**B**) Total numbers of colonic tumors per mouse (n = 20 [WT + Control diet]; 7 [WT + BHA]; 7 [WT + RSV]; 15 [*Mkp-1*$^{-/-}$ + Control diet]; 5 [*Mkp-1*$^{-/-}$ + BHA]; 6 [*Mkp-1*$^{-/-}$ + RSV]). Values are mean ± SEM; *P < 0.05, **P < 0.01. (**C**) Representative H&E staining of colonic tumor sections at the end of AOM/DSS treatment. a and d, Control diet; b and e, BHA supplementation; c and f, RSV supplementation. Scale bar, 100 µm. Original magnification × 40. T, tumor.

et al., 2018). For measurement of cDNA corresponding to mouse Nrf2 cDNA, the forward primer was 5′-GAGCCCTCTTTGTGATTCAGATTC-3′, the reverse primer was 5′-TTAGCCCTTCCAAACTACTCTAGGTATC-3′, and the probe was 5′-FAM-CACCAGCTCTTTGGAGTAATTGCTAA-TAMRA-3’. Each assay was performed in triplicate. The results were analyzed with 480II Real Time PCR System software (Roche, Shanghai, China). The level of 18S RNA was used as an internal standard.

2.6. Statistical analysis

Statistical analysis was carried out using Stata7 for Windows (StataCorp LLC, College Station, TX, USA). Student's t-test was used to compare two groups. Groups of more than two were compared by one-way ANOVA followed by Bartlett's test. Spearman's correlation was used to analyze two ranked variables. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Mkp-1 deficiency abolishes the anti-colitis effect of BHA and RSV

To test whether Mkp-1 is required for the anti-colitis effect of Nrf2 activators, BHA and RSV (200 mg/kg i.g.) were given to WT, *Mkp-1*$^{-/-}$, and *Nrf2*$^{-/-}$ mice that received DSS treatment. BHA and RSV reduced the severity of the colitis developed in the DSS-treated WT mice (Fig. 1), which exhibited a significantly longer colon (Fig. 1A, a and d) and reduced inflammation index (Fig. 1B, c and d; 1C). Inflammatory mediators such as IL-6, TNF-α and COX-2 are upregulated in UC{Khor et al., 2006 #18}. We found that BHA and RSV treatments caused lower levels of serum IL-6 and TNF-α (Fig. 2A), and lighter staining for nitrotyrosine, a marker of oxidative stress (Fig. 2B, c–d). The induction of COX-2 in the colon was also blocked by these compounds (Fig. 2C, lanes 3–4). As expected, dietary BHA and RSV were unable to elicit the beneficial phenotypic changes in *Nrf2*$^{-/-}$ mice (Figs. 1 and 2B, j − i). Strikingly, dietary suppression of colitis using BHA and RSV also failed in *Mkp-1*$^{-/-}$ mice (Figs. 1 and 2B, f − h). Our results demonstrated that, in addition to Nrf2, Mkp-1 is required for the action of BHA and RSV against inflammation and oxidative stress.

3.2. Mkp-1 deficiency increases susceptibility to colitis-associated adenoma

To assess the role of Mkp-1 in colitis-associated tumorigenesis, WT, *Mkp-1*$^{-/-}$, and *Nrf2*$^{-/-}$ mice were subjected to 6 weeks of AOM/DSS treatment to induce colitis-associated tumorigenesis. Although colonic tumors occurred across all three genotypes, the number of tumors per mouse was significantly higher in *Mkp-1*$^{-/-}$ mice (11.9 ± 4.2,

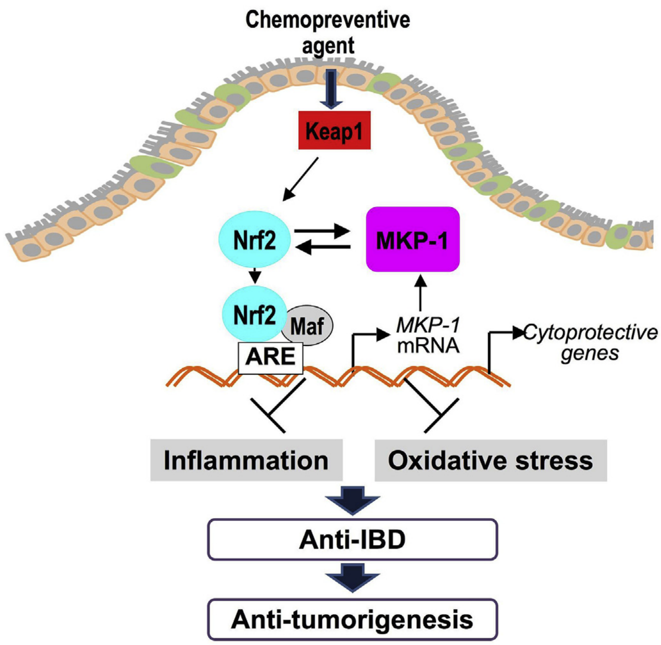

Fig. 6. Model for the role of Mkp-1 in colitis-associated colon tumorigenesis. Nrf2/ARE signaling is activated in response to chemopreventive agents and inflammatory stimuli. Inflammatory stimuli such as lipopolysaccharide induce the activation of Mkp-1 and Nrf2 transcription, while chemopreventive agents prevent Nrf2 protein degradation by modifying Keap1, and lead to the stabilization and activation of Nrf2. Upon activation, the Mkp-1 and Nrf2 proteins are translocated to the nucleus where they interact, leading to increased Nrf2 stability and the activation of ARE-driven genes including Mkp-1. Mkp-1 and Nrf2 form a feed-forward loop to protect colonic tissues against inflammation and prevent tumorigenesis.

$p < 0.01$) than in their WT counterparts (9.2 ± 3.8), while the number in *Nrf2*$^{-/-}$ mice was even higher (16.2 ± 5.1, $p < 0.01$) (Fig. 3A and B). Histological evaluation of tumors revealed that the majority from all three genotype were adenomas, which represent the initial stage of tumorigenesis (Fig. 3C, b-d). IHC revealed that although Ki-67 was induced in the colonic adenomas from all phenotypes, its expression was much higher in the adenomas from *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ mice (Fig. 3D, c and d) than in the WT (Fig. 3D, b), suggesting that tumor proliferation was more active in the knockout mice than in the WT.

3.3. Mkp-1 deficiency exacerbates inflammation and oxidative stress

The inflammatory response is thought to drive the development of tumors in the AOM/DSS model (Erreni et al., 2011; Isidro and Appleyard, 2016). IHC with antibody against F4/80, a marker of macrophages, revealed that the numbers of F4/80-positive cells were markedly higher in the colonic mucosa of *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ mice than in the WT after AOM/DSS exposure (Fig. 3D, f−h). Furthermore, the staining for nitrotyrosine and p53-binding protein 1 (p53BP1), which are markers of oxidative stress and DNA damage, respectively, was more intense in the colonic mucosa of *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ mice than WT mice after AOM/DSS exposure (Fig. 3E, b−d and f-h). These results suggest that, like Nrf2, Mkp-1 inhibits DNA damage and suppresses tumorigenesis by blocking inflammation and oxidative stress.

3.4. Reduced expression of Mkp-1, Nrf2 and genes downstream of Nrf2 in adenoma

We next assessed the expression of Mkp-1, Nrf2, and ARE-driven genes in adenomas from WT mice. The Mkp-1 and Nrf2 levels were markedly higher in adjacent normal tissue from WT mice that developed adenomas (Fig. 4A, lanes 4–6), than in the colonic tissue from WT mice without adenoma induction (Fig. 4A, lanes 1–3). However, in contrast to that in the adjacent tissue, the expression of these proteins was dramatically lower in the adenomas (Fig. 4A, lanes 7–9). Their mRNA levels in adenomas were also lower than in adjacent tissue (Fig. 4B). Accordingly, the mRNA and protein levels of the ARE-driven genes *Ho-1*, *Nqo1*, *Gclc*, and *Gstα1* were significantly lower in adenomas than in the adjacent normal tissue from WT mice with adenomas (Fig. 4C and D). Thus, our results clearly demonstrated that, while Mkp-1/Nrf2 signaling is up-regulated in adjacent normal tissue, it is down-regulated in colon adenoma, suggesting that loss of the cytoprotective system regulated by Mkp-1/Nrf2 is associated with the initiation of colitis-associated cancer.

3.5. Mkp-1 deficiency abolishes the chemopreventive effect of BHA and RSV

To investigate whether Mkp-1 is required for the chemopreventive effect of the Nrf2 activators, WT and *Mkp-1*$^{-/-}$ mice were given diets supplemented with BHA (0.5%) or RSV (300 ppm) while they were exposed to DSS/AOM to induce adenoma (Fig. 5A). As expected, the supplementation significantly reduced the number of adenomas developed in WT mice (Fig. 5B and C, b and c). However, the same supplementation failed to have any effect on the tumor development in *Mkp-1*$^{-/-}$ mice (Fig. 5B and C, e and f). Our data indicate that the tumor-suppression activity of BHA and RSV is dependent on Mkp-1.

4. Discussion

Our results showed that the action of the Nrf2 activators BHA and RSV in anti-colitis and the suppression of tumorigenesis is Mkp-1 dependent. We provide evidence for the first time that loss of Mkp-1 abolishes the chemopreventive effects of these agents. This study reveals a novel function of Mkp-1 in chemoprevention.

In the model of AOM/DSS-induced colitis-associated tumorigenesis, the phenotype of *Mkp-1*$^{-/-}$ mice was nearly identical to that of *Nrf2*$^{-/-}$ mice. Both the *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ mice had more tumors than WT mice in this model. IHC revealed enhanced epithelial proliferation in *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ tumors. This phenotype was associated with significant increases in infiltrating monocytes/macrophages with more proteins nitrosylated at the tyrosine residue after expression. Moreover, increased expression of p53BP-1 was found in the *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ tumors. These results suggest that inhibiting the oxidative stress and DNA damage caused by inflammation is a common mechanism for Mkp-1 and Nrf2 in protection against colitis-associated tumorigenesis.

It is recognized that Nrf2 plays dual roles during carcinogenesis: one is preventive during tumor initiation, and the other promotes progression (Satoh et al., 2013). In this study, we used a model of colitis-associated tumorigenesis induced by 6 weeks of AOM/DSS, representing an early stage of tumorigenesis. We found that the expression levels of Nrf2, Mkp-1 and ARE genes were higher in the adjacent normal tissue from WT mice compared to the control tissue, suggesting that adjacent normal tissues were under the stress of inflammation, which upregulates the Mkp-1/Nrf2 signaling. This observation is in consistent with our recent report that Mkp-1/Nrf2 signaling is activated in response to inflammatory stimuli{Li et al., 2018}. The higher expression of ARE-driven genes in the adjacent normal tissue suggests that a strong Mkp-1/Nrf2/ARE-controlled cytoprotective system in the mucosa is crucial for protection against the increased oxidative stress caused by inflammation in colitis. Strikingly, the expression levels of Nrf2 and its target genes in adenomas from WT mice were much lower than those in the adjacent normal tissue, further confirming the preventive role of the Nrf2/ARE system at the initiation of tumorigenesis. Interestingly, Mkp-1 expression was also lower in the adenomas, correlated with that of Nrf2 and ARE-driven genes. This result is consistent with our prior finding that Mkp-1 and Nrf2 form a forward feedback loop (Luo et al., 2018; Li et al., 2018), demonstrating the action of Mkp-1 in regulating the Nrf2/ARE system for protection against colonic inflammation. The higher expression of ARE-driven genes in the adjacent normal tissue suggests that a strong Mkp-1/Nrf2/ARE-controlled cytoprotective system in the mucosa is crucial for protection against the increased oxidative stress caused by inflammation in colitis. On the other hand, downregulation of this signaling may result in reduced host protection and greater susceptibility to damage by the oxidative stress caused by inflammation. Our results suggest that increased tumorigenesis may be driven by impairment of the Mkp-1/Nrf2 axis, and points to the importance of upregulating Mkp-1/Nrf2/ARE signaling in the prevention of colorectal tumorigenesis.

Pharmacological intervention with small-molecule activators of Nrf2 signaling provides protection against inflammation and prevents tumorigenesis (Rangasamy et al., 2004, 2005; Osburn et al., 2007; Thimmulappa et al., 2007; Ahmed et al., 2017). In agreement with previous reports that RSV and BHA have strong anti-inflammatory and chemopreventive properties (Reimund et al., 1998; Gescher et al., 2001; Greenwald et al., 2002; Martin et al., 2006; Cheung et al., 2010; Cui et al., 2010), we also found that they reduced the damage in DSS-induced colitis as well as inhibiting the AOM/DSS-induced tumorigenesis in WT mice. Intriguingly, neither BHA nor RSV supplementation had beneficial effects in *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ mice, confirming that, in addition to Nrf2, Mkp-1 is required for BHA- and RSV-induced chemopreventive effects. It is well established that Mkp-1 negatively regulates immune responses by deactivating mitogen-activated protein kinases (Liu et al., 2007). Further studies are ongoing to determine whether these kinases are implicated in the chemopreventive role of Mkp-1.

Taking these findings together, we propose a model in which Keap1, Mkp-1, and Nrf2 work together against colitis-associated tumorigenesis (Fig. 6). Keap1 serves as a key sensor for chemopreventive agents. Upon modification of Keap1, Nrf2 is de-repressed and hence accumulates in the nucleus. In response to inflammatory stimuli, Nrf2 activity is further enhanced by Mkp-1. Nrf2 induces the transcription of ARE-driven genes, including Mkp-1, leading to further upregulation of Mkp-1. Keap1, Mkp-1, and Nrf2 form a network in activating the expression of ARE-driven genes to limit excessive inflammation and to suppress tumorigenesis. Given the critical role of Mkp-1 in the progression of inflammation-mediated tumorigenesis, modulation of the Mkp-1/Nrf2 axis by small molecules may be a useful therapeutic strategy for the treatment of inflammatory bowel disease and the prevention of colorectal cancer.

Acknowledgements

We thank Prof. Masayuki Yamamoto (University of Tsukuba, Japan) for providing the *Nrf2*$^{-/-}$ mice, Bristol Myers Squibb Co. for permission to use the *Mkp-1*$^{-/-}$ mice, and Prof. John Hayes for providing antiserum against Gstα1/2. This work was supported by the National Natural Science Foundation of China, China (31571476, 91643110, 31470752, 31370772).

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.02.044.

References

Ahmed, S.M.U., Luo, L., Namani, A., Wang, X.J., 2017. Nrf2 signaling pathway: pivotal roles in inflammation. Biochim. Biophys. Acta 1863, 585–597.
Cheung, K.L., Khor, T.O., Huang, M.T., Kong, A.N., 2010. Differential in vivo mechanism of chemoprevention of tumor formation in azoxymethane/dextran sodium sulfate mice by PEITC and DBM. Carcinogenesis 31, 880–885.
Cui, X., Jin, Y., Hofseth, A.B., Pena, E., Habiger, J., Chumanevich, A., Poudyal, D., Nagarkatti, M., Nagarkatti, P.S., Singh, U.P., Hofseth, L.J., 2010. Resveratrol suppresses colitis and colon cancer associated with colitis. Cancer Prev. Res. 3, 549–559.
Dinkova-Kostova, A.T., Fahey, J.W., Talalay, P., 2004. Chemical structures of inducers of nicotinamide quinone oxidoreductase 1 (NQO1). Methods Enzymol. 382, 423–448.
Dinkova-Kostova, A.T., Talalay, P., 2008. Direct and indirect antioxidant properties of inducers of cytoprotective proteins. Mol. Nutr. Food Res. 52 (Suppl. 1), S128–S138.
Eaden, J.A., Abrams, K.R., Mayberry, J.F., 2001. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut 48, 526–535.
Elshaer, M., Chen, Y., Wang, X.J., Tang, X., 2018. Resveratrol: an overview of its anti-cancer mechanisms. Life Sci. 207, 340–349.
Erreni, M., Mantovani, A., Allavena, P., 2011. Tumor-associated macrophages (TAM) and inflammation in colorectal cancer. Cancer Microenviron 4, 141–154.
Friedman, S., Rubin, P.H., Bodian, C., Goldstein, E., Harpaz, N., Present, D.H., 2001. Screening and surveillance colonoscopy in chronic Crohn's colitis. Gastroenterology 120, 820–826.
Gescher, A.J., Sharma, R.A., Steward, W.P., 2001. Cancer chemoprevention by dietary constituents: a tale of failure and promise. Lancet Oncol. 2, 371–379.
Gescher, A.J., Steward, W.P., 2003. Relationship between mechanisms, bioavailability, and preclinical chemopreventive efficacy of resveratrol: a conundrum. Cancer Epidem Biomar 12, 953–957.
Greenwald, P., Milner, J.A., Anderson, D.E., McDonald, S.S., 2002. Micronutrients in cancer chemoprevention. Cancer Metastasis Rev. 21, 217–230.
Holtzclaw, W.D., Dinkova-Kostova, A.T., Talalay, P., 2004. Protection against electrophile and oxidative stress by induction of phase 2 genes: the quest for the elusive sensor that responds to inducers. Adv. Enzym. Regul. 44, 335–367.
Hu, R., Shen, G., Yerramilli, U.R., Lin, W., Xu, C., Nair, S., Kong, A.N., 2006. In vivo pharmacokinetics, activation of MAPK signaling and induction of phase II/III drug metabolizing enzymes/transporters by cancer chemopreventive compound BHA in the mice. Arch Pharm. Res. (Seoul) 29, 911–920.
Isidro, R.A., Appleyard, C.B., 2016. Colonic macrophage polarization in homeostasis, inflammation, and cancer. Am. J. Physiol. Gastrointest. Liver Physiol. 311, G59–G73.
Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M., Nabeshima, Y., 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem. Biophys. Res. Commun. 236, 313–322.
Kensler, T.W., Wakabayashi, N., 2010. Nrf2: friend or foe for chemoprevention? Carcinogenesis 31, 90–99.
Khor, T.O., Huang, M.T., Kwon, K.H., Chan, J.Y., Reddy, B.S., Kong, A.N., 2006. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. Cancer Res. 66, 11580–11584.
Khor, T.O., Huang, M.T., Prawan, A., Liu, Y., Hao, X., Yu, S., Cheung, W.K., Chan, J.Y., Reddy, B.S., Yang, C.S., Kong, A.N., 2008. Increased susceptibility of Nrf2 knockout mice to colitis-associated colorectal cancer. Cancer Prev. Res. 1, 187–191.
Krehl, S., Loewinger, M., Florian, S., Kipp, A.P., Banning, A., Wessjohann, L.A., Brauer, M.N., Iori, R., Esworthy, R.S., Chu, F.F., Brigelius-Flohe, R., 2012. Glutathione peroxidase-2 and selenium decreased inflammation and tumors in a mouse model of inflammation-associated carcinogenesis whereas sulforaphane effects differed with selenium supply. Carcinogenesis 33, 620–628.
Li, C., Xu, X., Wang, X.J., Pan, Y., 2014. Imine resveratrol analogues: molecular design, Nrf2 activation and SAR analysis. PLoS One 9, e101455.
Li, J., Wang, H., Zheng, Z., Luo, L., Wang, P., Liu, K., Namani, A., Jiang, Z., Wang, X.J., Tang, X., 2018. Mkp-1 cross-talks with Nrf2/Ho-1 pathway protecting against intestinal inflammation. Free Radic. Biol. Med. 124, 541–549.
Lippmann, D., Lehmann, C., Florian, S., Barknowitz, G., Haack, M., Mewis, I., Wiesner, M., Schreiner, M., Glatt, H., Brigelius-Flohe, R., Kipp, A.P., 2014. Glucosinolates from pak choi and broccoli induce enzymes and inhibit inflammation and colon cancer differently. Food Funct 5, 1073–1081.
Liu, Y., Shepherd, E.G., Nelin, L.D., 2007. MAPK phosphatases–regulating the immune response. Nat. Rev. Immunol. 7, 202–212.
Long, M., Tao, S., Rojo de la Vega, M., Jiang, T., Wen, Q., Park, S.L., Zhang, D.D., Wondrak, G.T., 2015. Nrf2-dependent suppression of azoxymethane/dextran sulfate sodium-induced colon carcinogenesis by the cinnamon-derived dietary factor cinnamaldehyde. Cancer Prev. Res. 8, 444–454.
Luo, L., Chen, Y., Wang, H., Wang, S., Liu, K., Li, X., Wang, X.J., Tang, X., 2018. Mkp-1 protects mice against toxin-induced liver damage by promoting the Nrf2 cytoprotective response. Free Radic. Biol. Med. 124, 541–549.
Luo, L., Chen, Y., Wu, D., Shou, J., Wang, S., Ye, J., Tang, X., Wang, X.J., 2015a. Butylated hydroxyanisole induces distinct expression patterns of Nrf2 and detoxification enzymes in the liver and small intestine of C57BL/6 mice. Toxicol. Appl. Pharmacol. 288, 339–348.
Luo, L., Chen, Y., Wu, D., Shou, J., Wang, S., Ye, J., Tang, X., Wang, X.J., 2015b. Differential expression patterns of Nqo1, AKR1B8 and Ho-1 in the liver and small intestine of C57BL/6 mice treated with sulforaphane. Data Brief 5, 416–423.
Martin, A.R., Villegas, I., Sanchez-Hidalgo, M., de la Lastra, C.A., 2006. The effects of resveratrol, a phytoalexin derived from red wines, on chronic inflammation induced in an experimentally induced colitis model. Br. J. Pharmacol. 147, 873–885.
Nair, S., Xu, C., Shen, G., Hebbar, V., Gopalakrishnan, A., Hu, R., Jain, M.R., Lin, W., Keum, Y.S., Liew, C., Chan, J.Y., Kong, A.N., 2006. Pharmacogenomics of phenolic antioxidant butylated hydroxyanisole (BHA) in the small intestine and liver of Nrf2 knockout and C57BL/6J mice. Pharmaceut. Res. 23, 2621–2637.
Osburn, W.O., Karim, B., Dolan, P.M., Liu, G., Yamamoto, M., Huso, D.L., Kensler, T.W., 2007. Increased colonic inflammatory injury and formation of aberrant crypt foci in Nrf2-deficient mice upon dextran sulfate treatment. Int. J. Cancer 121, 1883–1891.
Pandurangan, A.K., Saadatdoust, Z., Esa, N.M., Hamzah, H., Ismail, A., 2015. Dietary cocoa protects against colitis-associated cancer by activating the Nrf2/Keap1 pathway. Biofactors 41, 1–14.

Pascal, R.R., 1994. Dysplasia and early carcinoma in inflammatory bowel disease and colorectal adenomas. Hum. Pathol. 25, 1160–1171.

Pearson, W.R., Windle, J.J., Morrow, J.F., Benson, A.M., Talalay, P., 1983. Increased synthesis of glutathione S-transferases in response to anticarcinogenic antioxidants. Cloning and measurement of messenger RNA. J. Biol. Chem. 258, 2052–2062.

Rangasamy, T., Cho, C.Y., Thimmulappa, R.K., Zhen, L.J., Srisuma, S.S., Kensler, T.W., Yamamoto, M., Petrache, I., Tuder, R.M., Biswal, S., 2004. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. J. Clin. Investig. 114, 1248–1259.

Rangasamy, T., Guo, J., Mitzner, W.A., Roman, J., Singh, A., Fryer, A.D., Yamamoto, M., Kensler, T.W., Tuder, R.M., Georas, S.N., Biswal, S., 2005. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. J. Exp. Med. 202, 47–59.

Reimund, J.M., Allison, A.C., Muller, C.D., Dumont, S., Kenney, J.S., Baumann, R., Duclos, B., Poindron, P., 1998. Antioxidants inhibit the in vitro production of inflammatory cytokines in Crohn's disease and ulcerative colitis. Eur. J. Clin. Investig. 28, 145–150.

Rubiolo, J.A., Mithieux, G., Vega, F.V., 2008. Resveratrol protects primary rat hepatocytes against oxidative stress damage: activation of the Nrf2 transcription factor and augmented activities of antioxidant enzymes. Eur. J. Pharmacol. 591, 66–72.

Satoh, H., Moriguchi, T., Takai, J., Ebina, M., Yamamoto, M., 2013. Nrf2 prevents initiation but accelerates progression through the Kras signaling pathway during lung carcinogenesis. Cancer Res. 73, 4158–4168.

Siegel, R., Naishadham, D., Jemal, A., 2013. Cancer statistics, 2013. CA A Cancer J. Clin. 63, 11–30.

Singh, B., Shoulson, R., Chatterjee, A., Ronghe, A., Bhat, N.K., Dim, D.C., Bhat, H.K., 2014. Resveratrol inhibits estrogen-induced breast carcinogenesis through induction of NRF2-mediated protective pathways. Carcinogenesis 35, 1872–1880.

Smallie, T., Ross, E.A., Ammit, A.J., Cunliffe, H.E., Tang, T., Rosner, D.R., Ridley, M.L., Buckley, C.D., Saklatvala, J., Dean, J.L., Clark, A.R., 2015. Dual-specificity phosphatase 1 and tristetraprolin cooperate to regulate macrophage responses to lipopolysaccharide. J. Immunol. 195, 277–288.

Suzuki, R., Kohno, H., Sugie, S., Tanaka, T., 2004. Sequential observations on the occurrence of preneoplastic and neoplastic lesions in mouse colon treated with azoxymethane and dextran sodium sulfate. Cancer Sci. 95, 721–727.

Talalay, P., Batzinger, R.P., Benson, A.M., Bueding, E., Cha, Y.N., 1978. Biochemical studies on the mechanisms by which dietary antioxidants suppress mutagenic activity. Adv. Enzym. Regul. 17, 23–36.

Thimmulappa, R.K., Fuchs, R.J., Malhotra, D., Scollick, C., Traore, K., Bream, J.H., Trush, M.A., Liby, K.T., Sporn, M.B., Kensler, T.W., Biswal, S., 2007. Preclinical evaluation of targeting the Nrf2 pathway by triterpenoids (CDDO-Im and CDDO-Me) for protection from LPS-induced inflammatory response and reactive oxygen species in human peripheral blood mononuclear cells and neutrophils. Antioxidants Redox Signal. 9, 1963–1970.

Wang, X.J., Hayes, J.D., Wolf, C.R., 2006. Generation of a stable antioxidant response element-driven reporter gene cell line and its use to show redox-dependent activation of nrf2 by cancer chemotherapeutic agents. Cancer Res. 66, 10983–10994.

Wattenberg, L.W., 1985. Chemoprevention of cancer. Cancer Res. 45, 1–8.

Wu, X., Song, M., Gao, Z., Sun, Y., Wang, M., Li, F., Zheng, J., Xiao, H., 2017. Nobiletin and its colonic metabolites suppress colitis-associated colon carcinogenesis by down-regulating iNOS, inducing antioxidative enzymes and arresting cell cycle progression. J. Nutr. Biochem. 42, 17–25.

Yang, Y., Cai, X., Yang, J., Sun, X., Hu, C., Yan, Z., Xu, X., Lu, W., Wang, X., Cao, P., 2014. Chemoprevention of dietary digitoflavone on colitis-associated colon tumorigenesis through inducing Nrf2 signaling pathway and inhibition of inflammation. Mol. Canc. 13, 48.